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Research Article

Protein quality and antigrowth effect of protein isolate of Mucuna (*Mucuna Pruriens*) and Canavalia (*Canavalia ensiformis*) seeds

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Abstract: *Mucuna pruriens* and *Canavalia ensiformis* are legumes promoted by smallholder farmers in Africa. The beans contain high protein content but remain a minor food crop due to the presence of antinutrients. The potential for the utilization of Mucuna and Canavalia beans as an alternative source of protein was evaluated by isolating protein and assessing the effect of technique of their protein quality and antinutrient compounds. Protein quality was determined by *in vitro* and *in vivo* rat balance methodologies. Processing technique reduced total phenolics and tannins at about 50% and slightly improved *in vitro* Protein digestibility (IVPD) of both beans. True digestibilities for protein isolate of beans (60.39% Mucuna, 57.57% Canavalia) were not negligible. However, rats fed diets formulated with protein isolate from Mucuna and Canavalia lost weight, and the diets resulted in poor protein quality indices, negative value of PER (-1.33 and -2.36), and low values for NPER (0.38 and 0.73). This suggests that the antinutritive and toxic factors of raw bean of Mucuna and Canavalia were not eliminated efficiently during protein isolation. Since hydrothermal techniques have proved success on reduction of antinutrients, further study is envisaged to apply hydrothermal technique of isolating protein on Mucuna and Canavalia beans.
Keywords: Mucuna, Canavalia, Anti-nutrients, protein isolate, protein quality

INTRODUCTION

Food legumes are major sources of proteins in the population diets of many developing countries. In fact, the high cost of animal protein has deviated interest towards several leguminous seed as potential sources of vegetable proteins for human food and livestock feed. Since legume seeds are important sources of proteins, there has been a worldwide interest in searching for potential utilization of unconventional legumes [1].

Mucuna pruriens and *Canavalia ensiformis* are lesser known and underutilized tropical legumes which have not been fully utilized to alleviate the problem of protein malnutrition. Mucuna and Canavalia seeds are rich in proteins with values ranges 23-35% [2] and 28.9-35% [3] respectively. However their uses as the source proteins are limited by anti-nutritional factors such as antitrypsin factors, tannins, anticoagulants, phytates, 3,4-dihydroxy-L-phenylalanine (L-Dopa) and canavananin [4] [5]. The effects of anti-nutritional factors on body are known as the causes of poor proteins digestibility, reduce food intake, nutrients availability and can provoke deleterious effects on the many organs [6].

To improve protein quality of grain legume some processing techniques such as soaking, cooking, dehulling, roasting, fermentation, sprouting, toasting have been employed to reduce or destroy antinutrients. Many of these techniques were applied on Mucuna and Canavalia beans [7] [8] [9] [10]. But, they are always not effective [11] [12]. Techniques employed for extracting and isolating protein on grain legume are nowadays known to be effective in the elimination of the antinutrients [13] [14]. Generally, these techniques are employed to obtain protein concentrates or isolates. At the best of our knowledge, such study has not been applied to mucuna and canavalia.

Therefore, the aim of this study was to produce and investigate the protein quality of Mucuna and Canavalia beans isolates.

MATERIALS AND METHODS

Materials

Seeds of *M. pruriens* and *C. ensiformis* were purchased from local markets of Ngaoundere (Cameroon) and manually separated from infested seeds and impurities.

Preparation of Mucuna and Canavalia bean flours

The flours were produced from seeds legumes according to the method of Kaptso [15]. The seeds were soaked at ambient temperature for overnight in tap water with bean to water ratio of 1 to 10 (w/v). After soaking, seeds were dried for 24 h at 50°C and dehulled manually. The dehulled Seeds were grounded to flour

using a hammer Mill and sieved with the 500 µm mesh sieve and stored in polyethylene bags at 4°C until analysis.

Preparation of protein isolate of Canavalia and Mucuna bean flours

Mucuna and Canavalia beans proteins were isolated from flours according to Lawal [16] with some modifications. The powder was suspended in distilled water in the ratio of 1:5 (w/v) and stirred with an electric stirrer (TECHNICON, England) for 3h at 32°C. During the stirring process, suspension was adjusted to pH11 with 1M sodium carbonate. The suspension was centrifuged at 4000g for 30 min at 4°C, the supernatant decanted and the residue re-extracted twice under the same conditions. The supernatants were combined and the pH adjusted to 4.5 with 1 M citric acid to allow the proteins to precipitate for 5 min. Following this the proteins suspensions were centrifuged at 4000 g, 4°C for 30 min. Protein isolates were dried at 40°C for 24 h in an air electric ventilated oven, ground and passed through a 500 µm mesh sieve, packaged in polyethylene bags and stored at 4°C for further analysis.

Determination of proximate composition of flour and protein isolates

The moisture content [17] and the crude fat and total ash [18] were evaluated using standard methods. Crude fiber was estimated following the acid digestion procedure of Wolff [19]. Total nitrogen was determined after mineralization in concentrated sulfuric acid followed by colorimetric determination of ammonium according to Devani et al. [20] and the crude protein was calculated as nitrogen × 6.25.

Evaluation of Amino acids profile of crude flours

The amino acid compositions of crude flours were determined according to method of Spackman et al. [21] using an automated amino acid analyser after hydrolysing the samples with 6 N HCl at 105 °C for 24 hrs.

Antinutrients determination

Phytic acid was extracted in 1.2% HCl solution containing 10 % Na_2SO_4 [22] and quantified based on the formation of complex with Fe(III) ion at pH 1-2 according to the procedure of Stone et al. [23]. In this reaction an excess of Fe (III) ion present in the solution reacted with thiocyanate ion to form a characteristic pink complex, $Fe(SCN)_3$. The optical density at 465 nm was measured and an inverse linear relation was found with phytate concentration from 40 to 200 nmol/L.

Total phenolics content was determined as gallic acid equivalents [24] after extraction with 70% (v/v) alcohol [25]. In the same extract, total tannin content was determined by the precipitation method using polyvinylpyrrolidone (PVPP) as described by Makkar et al. [25]. PVPP in extract bind to tannins and make them inert. Briefly in 100 mg PVPP, 1.0 mL of distilled water and 1.0 mL of sample extract were added. The blend was vortexed and kept at 4°C for 15 min, vortexed once more and centrifuged at 3000 g for 10 min. The supernatant composed of only simple phenolics other than tannins were collected. The phenolic content of the supernatant were determined as mentioned above and the content of non-tannin phenolics expressed. The tannin content of the sample was calculated as difference:

Tannin (%) =Total phenolics (%) –Non-tannin phenolics (%).

***In vitro* protein digestibility determination**

Digestibility was determined using Yousif and Tinay method [26]. In the procedure, 0.2 g of the sample was placed in a 50 mL centrifuge tube, 15 mL of 0.1N HCl containing 1.5 mg pepsin were added, and the tube was incubated at 37°C for 3h. The suspension was then neutralized with 0.5 N NaOH then treated with 4 mg of pancreatin in 7.5 mL of 0.2M phosphate buffer (pH=8.0), containing 0.005M sodium azide; the mixture was then gently shaken and incubated at 37°C for 24h. After incubation the sample was treated with 10mL, 10% trichloroacetic acid, and centrifuged at 50,000 g for 20 min at room temperature and the supernatant was recovered. Nitrogen was estimated using the Kjeldahl method and digestibility expressed in percent was calculated as the ratio of the nitrogen in supernatant to that in sample.

Diet formulation

The experimental diets were prepared according to Vadde et al. [27] as shown in Table 1. Diets 3 and 4 were prepared using protein isolate of Mucuna bean and Canavalia as protein sources. Diet 2 was the standard diet using with casein as protein source while diet1 was the free protein diet.

Table1: Composition of the diets used in the experiments with rats (g/100g of the mixture)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Mineral mix	1	1	1	1
Vitamin mix	4	4	4	4
cellulose	5	5	5	5
Tournesol oil	10	10	10	10
Cassava starch	80	70	70	70
Casein	-	10	-	-
Mucuna beans protein isolate	-	-	10	-
Canavalia beans protein isolate	-	-	-	10

Diet 1: protein free diet, Diet 2: diet with casein, Diet 3: diet with protein isolate of Mucuna beans, Diet: diet with protein isolate of Canavalia beans.

Animal experiments and biological assay

Thirty-two male Wistar rats aged 22 -31 days weighing 42-62 g were obtained from Animal House of National school of Agro-Industrial Sciences. Animals were divided into 4 groups with eight animals each. The rats were placed in individual metabolic cages. After an acclimatation period of 7 days during which the rats were feed standard diet, each group of rats was fed on their experiment diets. The temperature of laboratory was 27±4 °C while the experiment alternate 12 h periods of light and dark. Rats received water and their experimental diets *ad libitum* for 14 days. Individual rat body weight, feed intake and feed waste were measured and recorded per two days and used in calculating days weight gain or loss, protein intake, Protein Efficiency Ratio (PER) per rat for each group and Net Protein efficiency Ratio (NPER) following Adrian et al. [28] method. The true (TD) and apparent (AD) digestibility [18] was determined by measuring the amount of nitrogen ingested in the diet, the amount eliminated in the feces, and the metabolic loss in the feces, which corresponds to the fecal nitrogen in the protein free group. At the end of the experiment, the feces were dried at 105°C for 24 h, cooled, weighed and ground in a food processor for the determination of nitrogen concentration by the Kjeldahl method. The samples were analyzed in triplicate. All the nutritional parameters were calculated as followed:

$$PER = \frac{Gain\ in\ body\ weight\ (g)}{Protein\ cosumed\ (g)}$$

$$NPER = \frac{Gain\ in\ body\ weight\ (g) + Loss\ in\ body\ weight\ of\ protein\ free\ diet}{Protein\ consumed}$$

$$TD = \frac{Ni - (NF1 - NF2)}{Ni} \times 100$$

$$AD = \frac{Ni - NF1}{Ni} \times 100$$

Ni = Nitrogen intake of animals fed the test diet.
NF1 = Nitrogen excreted in feces of animals fed the test diet.
NF2 = Nitrogen excreted in feces of animals fed the protein-free diet.

Statistical analysis

The values were presented as means with their standard deviation (± SD). The data were subjected to one factor analysis of variance (ANOVA) and Duncan's Multiple range test analysis using the Statgraphics software, version 5.0. The statistically significant difference was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate composition of flour and protein isolate of Canavalia and Mucuna beans

The chemical composition of flour and protein isolate of Mucuna and Canavalia beans are presented in Table 2. The crude protein content of Mucuna (30.4%) and Canavalia (22.6%) beans were higher than the range value 22.4-24.9% of commonly consumed legumes common bean (*Phaseolus vulgaris*), chick pea (*Cicer arietinum*), lentil (*Lens culinaris*) and pigeon pea (*Cajanus cajan*) [29]. According to Sridhar and Seena [30] the minimum seed proteins of Canavalia ranges from 22.4% to 24.9%. The protein content of our Mucuna bean sample was quite similar to 31.9 %

recently reported on the dehulled mucuna beans [2]. Variations in legumes seed proteins contents have been associated not only to the difference in species, but also to interaction between genetic and environment [2].

Protein content of protein isolate (85.3%) of Mucuna bean was comparable to 87.5% and 87.6% reported on the same variety by Udensi and Okoronkwo [31] and Akaerue and Onwuka [32], respectively. Similarly higher value (81.50%) of proteins content was reported on bambara proteins isolate [33], but much lower values were reported for oat (67.9-74.0%) and sweet lupin (67.1%) [34]. The protein content of protein isolate of Canavalia (63.81%) was less than those reported on most common beans mentioned above. The relatively low protein content of Canavalia protein isolate might be due to the loss of acid-soluble proteins during isoelectric precipitation or the retention of protein in the residue by complexation with other seed material. Chew et al. [35] demonstrated that from the 87% of sweet lupin protein solubilised, only 59% was recovered by isoelectric precipitation.

Mucuna and Canavalia are poor sources of fat. During protein isolation, about 80% of the fats are lost probably due to the non solubilisation in the aqueous solution of extraction. Similarly significant reduction ($p<0.05$) in fibers were observed in both cases. However no considerable change was observed in the ash content. The values observed in the present study were within the 2.9 - 5% range reported for many legume varieties [36-37]. These results showed that beans and protein isolate from the two beans are rich sources of minerals.

Anti-nutritional compounds of flour and protein isolate of Canavalia and Mucuna beans

The total phenolics and tannins contents of Mucuna and Canavalia were reduced to about 50% during the isolating process. Phenolic compounds, notably tannins are known to have ability to decrease digestibility by complexing with dietary proteins and to lower the activity of several digestive enzymes (e.g. a-amylase, trypsin, chymotrypsin, lipase) [38]. The loss of phenolic and tannins content might be due to leaching of phenols into extraction water during precipitation of proteins at acidic pH. Phytic acid in legumes has been reported to lower the nutritional value due to limiting the bioavailability of dietary minerals, essential trace elements and also proteins [39], [40]. Phytate content of our Mucuna flour sample was higher than the values 0.9 % and 0.86%, reported for white and black Mucuna varieties respectively [41], while the value in our Canavalia flour sample was within the range 0.48–1.092% reported earlier [30]. During proteins isolation, there was an increase in phytate level with values in Mucuna and Canavalia isolates of 1.87% and 1.35% respectively (Table 2). The increase in phytate probably resulted from its ability to complex to complex with proteins which co-precipitate at the isoelectrical point.

In vitro protein digestibility of flour and protein isolate of Canavalia and Mucuna beans

As shown in Table 2, the *in vitro* protein digestibilities (IVPD) of raw Mucuna and Canavalia seeds differed significantly ($p<0.05$) to that of their protein isolates counterparts. The IVPD of protein isolates were significantly ($p<0.05$) higher than those of flours. The values of IVPD in this study (38.60% for Mucuna and 30.69% Canavalia) were lower than the ranges 71.5-76.9% and 59-64% reported for Mucuna [42] and Canavalia beans flours respectively [43]. The difference observed might reflect the difference in the method used for the determination of the digested proteins. In fact we determined the IVPD based on the pepsin and pancreatin enzymes actions while others used the multienzyme system (trypsin, chymotrypsin, peptidase). The most important thing to consider in this study is not the individual value of each sample, but the effect of isolation on the IVPD. In this respect the low increase in IVPD observed after treatment suggested that during protein isolation, the antinutrients were not significantly reduced in order to enable protein attack by enzymes. Phytic acid, 3,4-dihydroxyphenylalanine (L-DOPA), as well as condensed tannins and polyphenols are known to interact with protein and form complexes. These interactions could decrease the solubility of proteins and increase the degree of cross-linking which resulted in impairment of protease access to peptide bonds [44].

Amino acid composition of seeds of *C.ensiformis and M. pruriens*

The amino acid compositions of Canavalia and Mucuna beans flours and the essential amino acid requirements pattern suggested by FAO/WHO [45] are shown in table 3. The amino acid profiles of Mucuna beans revealed that the proteins seeds contained higher levels of some essential amino acid (Isoleucine, leucine, histidine, valine, threonine) compared to FAO/WHO reference. Sulphur-containing amino acids, cystine and methionine are the essential amino acid with values below the FAO reference. Usually sulphur-amino acids are the limiting amino acid in legumes proteins [46]. Aspartic and glutamic acids were predominant in Mucuna beans, results which were consistent to those reported by Mary and Janardhanan [47] and Siddhuraju et al. [48] in Mucuna seeds. Similarly histidine, Glutamic acid and aspartic acid were the major amino acids in canavalia. The essential amino acid of Canavalia (Isoleucine, leucine, histidine, valine, threonine) were higher than FAO/WHO reference and common legumes (*V. mungo and V. radiata, C. arietinum and C. cajan*) ([49]. Sulphur amino acids in Canavalia were close to the FAO/WHO reference and in this respect Canavalia protein could be considered as a legume source of amino acids.

Table 2: Proximate composition, antinutritional factors (g/100g dry weight basis) and *In vitro* protein digestibility (%) of flour and protein isolate of Mucuna and Canavalia beans

	M. pruriens		***C. ensiformis***	
Components	**Flour**	**Protein isolate**	**Flour**	**Protein isolate**
Moisture	5.59±0.28[a]	8.97±0.13[b]	3.40±0.14[a]	9.91±0.60[b]
Crude protein	30.45±0.15[a]	85.28±0.15[b]	22.59±0.68[a]	63.81±0.13[b]
Crude lipid	7.24 ±0.63[a]	2.02 ±0.3[b]	8.64 ± 0.59[a]	3.01±0.30[b]
Crude fibre	5.96±0.56[a]	1.70±0.7[b]	3.99±0.23[a]	1.39±3.7[b]
Ash	3.32±0.15[a]	3.12±0.13[b]	2.75±0.00[a]	3.72±0.75[a]
Total phenolics	4.65 ± 0.20[a]	2.62±0.21[b]	1.12±0.09[a]	0.37±0.02[b]
Tannins	2.04±0.34[a]	1.99 ± 0.15[b]	0.48± 0.11[a]	0.02± 0.00[b]
Phytate	1.18±0.08[a]	1.87±0.09[b]	0.98±0.10[a]	1.35±0.05[b]
IVDP (%)	38.60±0.29[a]	43.23±0.42[b]	30.69±0.36[a]	35.47±0.01[b]

Means ±SD (n=3) within each legume variety (*M. pruriens* or *C. ensiformis*), followed by different letters (a-b) in the same line are significantly different ($p<0.05$).

Table 3: Amino acid composition of Canavalia and Mucuna beans

Amino acids	*M. pruriens*	*C. ensiformis*	FAO/WHO Pattern (1991)
Essential amino acids (EAA) (mg/100g protein)			
Isoleucine	5.54	5.22	2.8
Leucine	8.42	8.07	6.6
Lysine	4.82	4.43	5.8
Histidine	4.54	14.07	1.9
Valine	6.21	6.27	3.5
Threonine	5.11	3.52	3.4
Phenylalanine Tyrosine	5.54[a]	4.57[a]	6.3[a]
Methionine Cystine	0.92[b]	1.95[b]	2.5[b]
Tryptophan	ND	ND	11
Non essential amino acids (NEAA) (mg/100g protein)			
Alanine	7.09	8.95	/
Proline	8.29	5.98	/
Arginine	6.67	7.25	/
Serine	6.02	5.94	/
Glycine	9.66	10.53	/
Aspartic acid	14.54	10.98	/
Glutamic acid	13.52	9.48	/

a: Phenylalanine + tyrosine, b : Cystine+Methionine, EAA: essential amino acid, NEAA: no essential amino acid.

Protein quality of protein isolates of Mucuna and Canavalia beans

As shown in table 4, rats fed Mucuna and Canavalia protein isolates exhibited significant lower food intake as compared to animal fed casein diet. The significant lower food intake in rats fed with the protein isolates than in control rats was probably due to the effects of antinutritional factors which remained in the protein isolates and consequently reduce the appetite of rats. The results presented in Figure 1 show that during the two week of experimentation, rats fed protein isolates diet lose weight. The protein true digestibility (TD) of Mucuna and Canavalia isolates showed trends similar to that of apparent digestibility (AD). However, TD values were higher than AD values, indicating higher absorption of nitrogen in protein isolates rats fed groups. AD and TD of Mucuna and Canavalia protein isolates were as expected inferior to casein fed rats group. In order words, the nitrogen absorbed from proteins isolates was lower compared to nitrogen from casein. The lost in weight observed on rats groups fed Mucuna and Canavalia isolated is then a consequence of poor protein quality indices, such as the negative value of PER, low values of NPER, AD and TD.

Feeding study by others researchers showed growth depression in experimental animals fed diets containing unprocessed Mucuna and Canavalia beans [50], [51], [43]. These reports attributed the growth depression to antinutrients and toxic factor components which tended to impair protein utilization, thereby reducing the nutritional value of the seeds protein. Isolation of proteins was expected in this study to reduce the antinutrients and induce rat's growth as compared to casein as reference proteins. This was not the case and the method of proteins isolation need to be improved. For instance it has been demonstrated that hydrothermal processing not only concentrated or isolated proteins, but also destroyed and reduced the level of antinutrients [52]. However the conditions under which this is feasible need to be fully investigated since such study has not been carried out on mucuna.

Table 4: Protein Efficiency Ratio (PER), Net Protein efficiency Ratio (NPER), food intake, apparent (AD) and True (TD) digestibility of casein, protein isolate of Mucuna and Canavalia beans

	Food intake (g)	PER	NPER	AD (%)	TD (%)
Casein	17.8 ±1.7[a]	3.71±0.11[a]	4.70±0.13[a]	82.99±0.19[a]	89.99±0.01[a]
Mucuna	12.6±1.4[b]	-1.33±0.26[b]	0.98±0.18[b]	41.50±0.01[b]	60.39±0.02[b]
Canavalia	11.4±1.3[b]	-2.36±0.69[c]	0.73±0.17[b]	40.43±0.3[c]	57.57±0.29[b]

Values are expressed as mean ± SD, n = 8 in each group. Means followed by different letters (a-c) in the same column are significantly different at $p<0.05$.

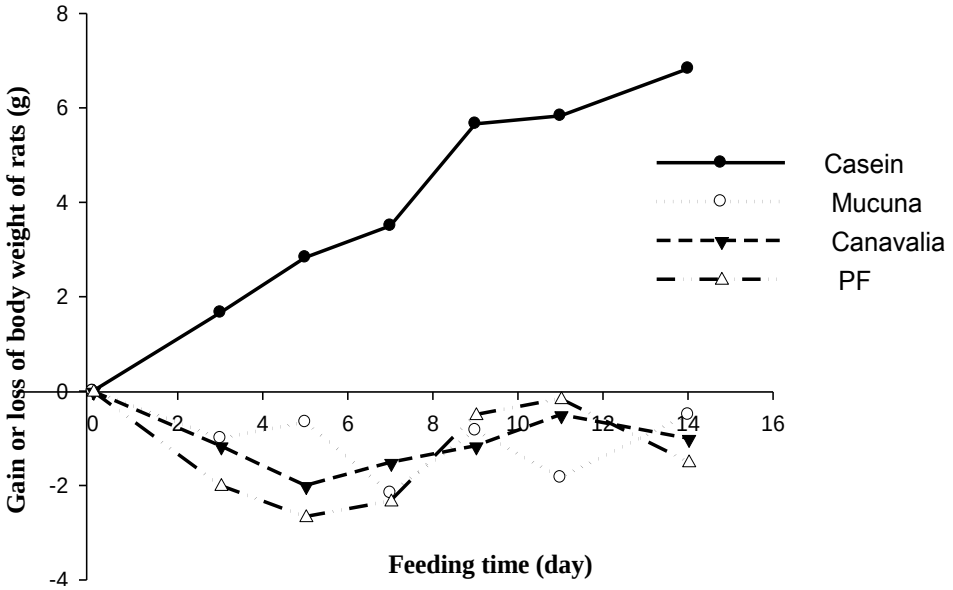

Figure 1: Evolution of gain or loss of body weight of rats groups (n=8) fed diets formulated with different proteins sources: casein, Mucuna and Canavalia proteins isolates and protein free (PF) diet.

CONCLUSION

Proximate composition of Mucuna and Canavalia beans compared favorably with that of conventional edible legumes. Consumption of protein isolates of Mucuna and Canavalia bean by weanling rats caused weight lost and their protein quality indices were poor. Improved isolating proteins techniques, such as the hydrothermal process should be envisaged to significantly reduce the antinutrients and toxic factors of mucuna and canavalia seeds, and hence improve the nutritional value of the proteins.

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